ing, was employed. Nevertheless residual lipids content was reduced to 1.68% oil at 3.8% moisture, or 1.58% oil calculated to 10% moisture.

Conclusions

The influence of certain variables upon the direct, continuous, solvent extraction of sunflower seed meats has been discussed. It has been shown that the ideal preparation consisted of first reducing the hull content to about 10% by weight. Then the dehulled meats were rolled, cooked, crisped, and re-rolled prior to solvent extraction. Drying before rolling, followed by cooking and crisping, was also helpful in the reduction of residual lipids content but to a lesser degree than was re-rolling after cooking.

It has been shown that the use of relatively low solvent/meats ratios required initially high mass velocities and that mass velocity during extraction was higher than that realized during cake washing.

The full industrial-scale run confirmed the experimental findings; thus the direct, continuous, solvent extraction of sunflower seed meats is a commercial reality. An oil-content reduction from 53% to 1.68% in one operation is a technological advance of great magnitude.

Except for periods when rice bran is processed, the plant is in continuous operation on sunflower seed meats and consistently produces meals containing between 1.5% and 1.7% oil content.

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Ozonolysis as a Method for Establishing the Position of Olefinic Linkages

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OZONOLYSIS combined with chromatography has not been an entirely satisfactory method in our laboratory for locating the positions of double bonds in unsaturated fatty acids. Literature reports (1, 2, 3, 4, 5, 6), including some which appeared after this work was begun, had indicated a reasonable degree of success with the method. We therefore examined the procedures in greater detail, using oleic acid as the representative compound.

The methyl oleate employed in our investigation satisfied the requirements for high purity on the basis of melting and freezing curves and infrared spectra. The presence of methyl linoleate was not detected in its ultraviolet spectra, and both iodine and saponification values lay well within the accepted limits of purity. Because the methyl oleate was converted to oleic acid under saponification conditions which previously had been shown to effect no conjugation of the double bond system of methyl linoleate, it was assumed that no isomeric octadecenoic acids were formed during saponification. When oleic acid was ozonized however and the products of ozonide decomposition were chromatographically resolved, relatively large fractions corresponding to seven-, eight-, and ten-carbon mono- and dicarboxylic acids were obtained in addition to the expected pelargonic and azelaic acids.

This report is concerned with our attempts to determine, in a semi-quantitative manner, the origin of these unexpected cleavage products. These acids might have been formed during either ozonization or ozonide decomposition or might have resulted from positional isomers contained in the methyl oleate which were not detected by the methods employed to establish its purity. Our results are summarized in Table I. Observations and conclusions regarding fractions other than the C_9 follow.

C_7 Dicarboxylic Acid Fraction. Runs 1, 2, 3, and 4 are representative of ozonizations carried out in solvents commonly used for this purpose; a relatively large C_7 dicarboxylic fraction was obtained in each case. It has been shown however that this fraction results from lower-molecular-weight monocarboxylic acids, such as acetic acid or other acidic substances. Thus, when acetone was treated with ozone, then heated with alkaline peroxide, and the product was chromatographically resolved, a broad peak in the region of the C_7–C_8 fraction was obtained. Similar results were also obtained with methanol and tetrachloromethane. n-Hexane was not attacked by ozone under the conditions employed, and its use as a solvent eliminated the C_7 fraction completely (Runs 6, 7, 8, and 11). Thus the C_7 fraction was an artifact and not pimelic acid.

C_{10} Dicarboxylic Acid Fraction. The C_{10} fraction, isolated by the same chromatographic procedure used in the analytical procedure, contained some azelaic acid and an unidentified amorphous substance but not sebacic acid. When the ozonide was subjected to vigorous decomposition (heated under reflux for 3 hrs. with alkaline peroxide, Run 11) or when ozonization was effected in acetic acid at 90–95° (at which temperature the ozonide probably decomposed as rapidly as it formed, Run 12), the C_{10} fraction was

TABLE I

Run	Compound	Solvent	Reagent	Method of decomposition	% Apparent recovery	% Dicarboxylic				% Monocarboxylic		
						10	9	8	7	10	9	8
1	Oleic	Acetic	O_3–O_2	Boiled in water 1 hr., re-ozonized 5 min.		1.3	33.5	5.8	59.4	6.2	83.0	10.6
2	Oleic	Acetic	O_3-Air	Boiled in water 1 hr., re-ozonized 20 min.			79.8	9.5	11.2	13.3	76.0	10.7
3	Oleic	CCl_4	O_3–O_2	Boiled in water 1 hr., re-ozonized 10 min.			79.0	9.3	11.5	12.1	78.5	9.4
4	Oleic	Acetone	O_3–O_2	Boiled with 1.0 *N* NaOH and 12% H_2O_2 for 45 min.		5.1	66.9	7.1	21.5			
5	Oleic	Hexane	O_3–O_2	Boiled with 1.0 *N* NaOH and 12% H_2O_2 for 50 min.	51.4	13.9	79.4	8.4				
6	Oleic	Hexane	O_3–O_2	Boiled in water 1 hr., re-ozonized 15 min.	66.0	1.8	88.1	9.8				
7	Oleic	Hexane	O_3–N_2	Boiled with 1.0 *N* NaOH and 30% H_2O_2 for 1 hr.	81.2	4.6	90.2	5.0		9.0	87.0	3.8
8	Methyl oleate	Hexane	O_3–N_2	Boiled with 1.0 *N* NaOH and 30% H_2O_2 in ethanol for 2.5 hrs.	70.8	4.6	87.5	6.8		10.3	83.0	6.6
9	1-Decene	Hexane	O_3–O_2	Allowed to stand with 1.0 *N* NaOH and 30% H_2O_2 for 2 hrs. Excess destroyed with CH_2O.	12.7						100.0	
10	1-Decene	Hexane + trace acetic	O_3–O_2								100.0	
11	Oleic	Hexane	O_3–O_2	Boiled with 1.0 *N* NaOH and 30% H_2O_2 for 3 hrs.	16.5					12.9	87.2	
12	Oleic	Acetic	O_3–O_2	Ozonized at 90–95°C. for 30 min. No subsequent decomposition step.	61.2		91.0	8.9		7.6	79.5	14.1
13	Pelargonic	Acetic	O_3–O_2	Ozonized at 90–95°C. for 45 min. No subsequent decomposition step.	64.7		94.2	5.8			91.8	8.2
14	Pelargonic	Acetic	O_3–O_2	Ozonized at 90–95°C. for 85 min. No subsequent decomposition step.	72.0						90.4	9.6
15	Oleic		$KMnO_4$–KIO_4				100.0			8.3	91.5	

eliminated. From these observations it was concluded that the C_{10} fraction was a partially decomposed ozonide which appeared in the same position as that in which sebacic acid would appear. Apparently the ozonide of oleic acid is more stable than had been assumed previously. This conclusion is in agreement with the fact that the stability of ozonides increases with increasing molecular weight (7).

C_8 Monobasic Acid Fraction. The possibility that the C_8 monobasic acid fraction, presumably containing caprylic acid, could result from peroxidation of the methylene adjacent to the 9-double bond in oleic acid (8) was recognized. Results obtained from Runs 7 and 8 however eliminated this possibility from consideration for ozonizations carried out in the absence of oxygen (9) failed to eliminate the C_8 monobasic acid fraction. Nor was it found as a product of the ozonization of pure 1-decene (Runs 9 and 10).

These observations coupled with the fact that vigorous ozonide decomposition (Run 11) eliminated the C_8 monobasic acid fraction led us to conclude that this fraction, like the C_{10} dicarboxylic acid fraction, was an artifact. The appearance of the C_8 monobasic acid fraction, but not the C_{10} dicarboxylic acid fraction among the products obtained when oleic acid was ozonized in acetic acid at 90–95°, appeared to contradict these observations and conclusions. This apparent inconsistency however was explained by the following observations. The products obtained from the ozonization gave a positive peroxide test just prior to chromatographic resolution, but those from vigorous alkaline peroxide degradation of the ozonide did not. Pelargonic acid was subjected to ozonization in acetic acid at 90–95° for varying periods of time (Runs 13 and 14), and resolution of the products yielded both the expected C_9 and the C_8 fractions. The size of the latter fraction did not increase when the time of ozonization was doubled, a condition to be expected if pelargonic acid were being converted to caprylic. The fraction gave a positive test for peroxide when treated with acidulated potassium iodide solution. The C_8 monobasic acid fraction apparently contained perpelargonic acid and not caprylic acid.

C_8 Dibasic Acid Fraction. The C_8 dibasic acid fraction was isolated, and from it a crystalline material was obtained. This was proved to be suberic acid by determination of its mixed melting point with an authentic sample. The possibility that it resulted from oxidative degradation or migration of the double bond during ozonization was excluded since no caprylic acid was detected as an oxidation product of 1-decene (Runs 9 and 10), which would be the case if double-bond migration or oxidative degradation occurred. Also an attempt to oxidize azelaic acid with alkaline peroxide was unsuccessful.

C_{10} Monobasic Acid Fraction. No attempt was made to isolate capric acid because of its low melting point and because of the difficulties involved in its isolation. The fraction corresponding to it however was always present among the ozonization products of oleic acid irrespective of the procedure by which the ozonide was decomposed. The C_{10} fraction was also obtained when oleic acid was oxidized by the permanganate-periodate method (10, 11). Failure to isolate suberic acid, the corresponding dibasic acid oxidation fragment, in this case resulted probably from its greater solubility in the large volume of aqueous solution employed. We have concluded therefore that the C_{10} monobasic and C_8 dibasic acid fractions consisted of capric and suberic acids, respectively, and that these acids originated from 8-octadecenoic acid present in the oleic acid in amounts which were not detected by the methods employed to establish the purity of oleic acid.

Recovery of Products from Ozonization. Ozonization was allowed to proceed to completion in all cases. The percentage of recoveries of acid products based on the quantities of the sample ozonized varied with the procedure used for ozonide decomposition and were generally quite low, as indicated by the values in Table I. These values are expressed as percentage of the theoretical total of titratable acidity. No values are given for Runs 1 through 4 since we have demonstrated that acidity is produced by oxidation of the solvents. It should be mentioned that the percentage values of the mono- and dibasic acid fractions were calculated on the amount of recovered acidic products rather than on the weight of original sample. The low recovery of acidic products was caused probably by secondary reactions of ozonides, which formed nonacidic or high-molecular-weight condensation products. Rieche (12) reported the formation of polyperoxide materials as secondary products of the ozonization of oleic acid, and Keppler (5) also found significant amounts of nonacidic material along with the acidic ozonization products of methyl oleate. The formation of these secondary products is a serious deterrent to the use of ozonization as a quantitative procedure.

Experimental

Preparation of Methyl Oleate. To a solution of 500 g. of olive oil in 2,000 ml. of anhydrous methanol and 1,500 ml. of diethyl ether were added 70 ml. of 0.5 *N* alcoholic potassium hydroxide. The mixture was gently heated until it became homogeneous and was then allowed to stand over-night at room temperature.

On the following day the mixture was agitated with an equal volume of water, and the water layer containing alcohol and soap was discarded. The organic layer containing methyl oleate was washed with water to remove any residual soap, was dried over anhydrous sodium sulfate, filtered, and freed of ether by distillation under vacuum.

The methyl ester was dissolved in acetone (500 g. of the ester in 5,000 ml. of acetone) and cooled to 25–30°, at which temperature the saturated esters crystallized and were removed by filtration. The filtrate was cooled to −60°, and the crude methyl oleate which crystallized was removed by filtration. It was redissolved in sufficient acetone to yield a 10% solution, from which methyl oleate was recrystallized at −55° and separated from the mother liquors by filtration. After removal of residual acetone by evaporation under vacuum, the methyl oleate was fractionally distilled under reduced pressure. Methyl oleate so obtained possessed a boiling point of 155° (0.5 mm.) and an iodine value of 86.4 (theoretical, 85.7); on the basis of infrared spectra it contained no *trans* double bond. Its cooling and melting curves likewise indicated a satisfactory degree of purity: f.p. $\alpha - 13.7°$, m.p. $\beta - 9.8°$. No polyunsaturation was present as determined by the Ultraviolet Spectrophotometric Method (A.O.C.S. Cd 7-58).

Saponification of Methyl Oleate. Methyl oleate (3.0 g.) was dissolved in 50.0 ml. of 0.5 *N* alcoholic potassium hydroxide, and the resulting solution was boiled under reflux for 30 min., then cooled and treated with water. After extraction with petroleum ether the aqueous solution was acidified with 1:1 aqueous hydrochloric acid solution, and the oleic acid was extracted with diethyl ether. The ether solution was washed several times with water to remove hydrochloric acid, then dried over anhydrous sodium sulfate, after which the ether was removed by evaporation at room temperature under vacuum. The resulting oleic acid was maintained under an atmosphere of nitrogen until needed.

Procedure for Ozonization. Ozonization was carried out by allowing a slow stream of oxygen (or air) and ozone, or nitrogen and ozone, to pass through a solution of the compound until the exit gas produced iodine when conducted through aqueous potassium iodide.

Preparation of Ozone-Nitrogen Mixture. A mixture of pure ozone in nitrogen was produced according to a modification of the method of Cook (10). The product of the ozone generator (95% oxygen and 5% ozone) was passed through 100 g. of activated silica gel (Davison 13–20 mesh) contained in a trap cooled to −40°. After absorption of the desired quantity of ozone, calculated from the ozone content of the generator product and the volume of gas which passed through the absorbent, the temperature of the trap was gradually raised and the ozone was allowed to desorb into a stream of dry nitrogen. The ozone-nitrogen mixture was conducted directly into the solution to be ozonized.

Conversion of Ozonization Products to Acids. Runs 1, 2, 3, 6. After ozonization, the solution containing the products was treated with 8 ml. of water and heated under reflux for 1 hr., then cooled and retreated with ozone for 5 to 20 min.

Runs 4, 5, 7, 8, 9, 10, 11. After ozonization the solution was treated with approximately 20 ml. of 1.0 *N* NaOH and 3–5 ml. of 30% hydrogen peroxide. In certain experiments the mixture was allowed to stand for a time (with occasional shaking), then was heated under reflux for varying times. During the period of heating both hydrogen peroxide and sodium hydroxide were added to maintain peroxide content and alkalinity. After removal of the solvent, heating was sometimes continued for 2–4 hrs. In some cases excess peroxide was destroyed by adding formaldehyde (18%) under alkaline conditions until a drop of the reaction mixture no longer gave a positive iodine test with acidic potassium iodide solution.

After treatment by one of the above two methods, the resulting reaction mixture was concentrated to dryness under vacuum, and the residue was treated with slightly more than the equivalent amount of 1.8 *N* hydrochloric acid (to convert the salts to fatty acids). The acids were extracted with diethyl ether, and the ether was evaporated. The mixture of acids so obtained was resolved directly into mono- and dicarboxylic acid fractions by means of the extraction procedure described below (Runs 1, 2, 3), or it was first dissolved in a mixture composed of 0.5 ml. *tert*-butyl alcohol and 3.0–5.0 ml. of chloroform. The volume then was increased to 50.0 ml. by addition of chloroform. An aliquot portion of this solution was analyzed chromatographically in the manner described below without separation into mono- and dicarboxylic acid fractions (Runs 4, 5, 6, 7, 8, 11, 15).

Run 12. The acetic acid was removed under vacuum, and the residue was analyzed chromatographically. A sample of the ozonization product treated with acidified potassium iodide solution gave a positive peroxide test.

Ozonization of Pelargonic Acid. Runs 13, 14. Pelargonic acid (1.097 g.) dissolved in 25 ml. of glacial acetic acid was treated with ozone at a temperature of 90–95° for a period of 45 min. At this point one-half of the solution was withdrawn, the acetic acid was removed under vacuum, and the residue was chromatographically analyzed in the usual manner.

The second half was treated with ozone at 90–95° for an additional 40 min., after which the acetic acid was removed and the residue was chromatographically analyzed. Both samples gave a positive test for peroxide when treated with acidulated potassium iodide solution.

Treatment of Acetone with Ozone. Twenty-five ml. of acetone were treated with ozone for 15 min., then with alkaline hydrogen peroxide as described under the procedure for converting ozonization products to acids. Chromatographic analysis of the product resulted in a broad peak in the region corresponding to pimelic and suberic acids. Methanol and tetrachloromethane yielded similar results.

Purification of n-Hexane. Fifty ml. of *n*-hexane (Phillips Technical Grade) were treated with ozone for 20 min. to remove any oxidizable material, after which it was heated under reflux with a mixture of 15 ml. of 1.0 *N* sodium hydroxide and 5 ml. of 12% H_2O_2 for 45 min. It was then washed once with water and dried over anhydrous calcium chloride.

Attempt to Isolate Sebacic Acid. Oleic acid, 1.9 g., was ozonized, and the products were prepared for chromatographic analysis in the manner described in II above. The entire sample however was transferred to a silicic acid column buffered to pH 5.5 and manually eluted with butanol-chloroform mixtures, as indicated below. The progress of elution was followed by titration of the fractions, and those fractions corresponding to sebacic acid were combined. The water was removed by distillation under vacuum. The residual salts were converted to acids by treatment with slightly more than the equivalent quantity of hydrochloric acid, and the acids (0.1 g.) were sublimed under a pressure of 0.01 mm. at 97°. A sample of the crystalline sublimate (m.p. 106°) was proved to be azelaic acid by determination of its mixed melting-point with an authentic sample. No higher-melting acid corresponding to sebacic was obtained. The residue in the sublimation apparatus remained unsublimed even at 135°.

Isolation of Suberic Acid. By means of the same procedure the suberic acid fraction from the ozonization of oleic acid was isolated, recrystallized, and sublimed. The sublimate melted at 132–134°. Its mixed melting-point with an authentic sample of suberic acid (138–139°) was undepressed at 132–134°.

Attempted Degradation of Azelaic Acid. Azelaic acid (0.50 g.) was heated under reflux with alkaline peroxide for 1 hr. From time to time aqueous hydrogen peroxide was added to maintain the concentration of oxidizing agent. The products of this treatment were recovered and analyzed chromatographically. The recovery of azelaic acid amounted to 96%, and no other acid was detected.

Chromatographic Procedure. After removal of the solvent by distillation, the mixture of mono- and dibasic acids and water was treated with 40 ml. of 1:4 v/v water-methyl Cellosolve solution, and the resulting solution was continuously extracted for 6–8 hrs. with Skellysolve F. Dibasic acids remained in the aqueous layer, which was made up to 50 ml. by addition of methyl Cellosolve. The Skellysolve fraction containing monobasic acid was likewise made up to 50 ml. (with Skellysolve).

Analysis of the mixture of dibasic acids (with or without separation from monobasic acids) was accomplished by introducing an aliquot of the aqueous Cellosolve solution onto a column consisting of 25 g. of silicic acid buffered with 25 ml. of a 1.0 *M* citrate buffer at pH 5.5 Elution was effected with an apparatus which continuously added a chloroform-butanol solution in which the percentage of butanol was automatically increased from 3 to 35% during the course of elution. The eluate was automatically collected in 7-ml. fractions, which were titrated with 0.02 *N* alcoholic potassium hydroxide. Phenolphthalein was used as an indicator, and the fraction was agitated during titration with a stream of nitrogen.

Analysis of the monobasic acids (with or without separation from dibasic acids) was carried out in the same way but with the silicic acid buffered with 2.0 *M* glycine at pH 10.1.

Summary

Ozonolysis and chromatographic procedures as a method for determining the position of double bonds in olefinic acids are not entirely satisfactory. Ozonolysis is seriously limited because of the secondary reactions undergone by the ozonide. Chromatographic resolution of the decomposed ozonides of oleic acid yields the expected pelargonic and azelaic acids but also yields fractions that indicate double bonds which do not exist. Fractions corresponding to pimelic, sebacic, caprylic, suberic, and capric acids appeared. The "pimelic acid" fraction resulted from the reaction of ozone with an oxidizable or hydrolyzable solvent such as acetone, methanol, or tetrachloromethane. The "sebacic" and "caprylic acid" fractions were incompletely decomposed ozonide. The suberic and capric acid fractions however were real and are believed to have resulted from 8-octadecenoic acid present in the oleic acid in amounts too small to be detected by the methods used to establish its purity.

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Erratum

Inadvertently the printer omitted four lines at the top of page 400 in the September 1959 issue, volume 36: "ess was conducted at 100°F.(38°C.). with furfural containing 2.5% of water. The cost of conducting the separation on a large scale is believed to be favorable enough to make the process commercially attractive." (Linoleic Acid from Safflower Oil by Liquid-Liquid Extraction, by Beal and Brekke.)